



Pharmacokinetic Study on Levodopa and Benserazide Hydrochloride Tablets in Beagle Dogs by HPLC-ESI-MS/MS

Xiao-Yan ZHANG ^{1,2#}, Wei ZHOU ^{3#}, Jing YANG ^{1*} & Geng-Li DUAN ^{2*}

¹ Department of Pharmacology, School of Basic Medical Sciences,
Wuhan University, Wuhan 430071, China

² Department of Pharmaceutical Analysis, School of Pharmacy,
Fudan University, Shanghai 201203, China

³ Department of Pharmaceutical Analysis, School of Pharmacy,
Guiyang Medical University, Guiyang 550004, China

SUMMARY. The simultaneous determination of levodopa and benserazide in Beagle dog plasma was presented by HPLC-ESI-MS/MS method applied to actual pharmacokinetic study using Beagle dogs. Effective chromatographic separation was achieved by using a C18 analytical column with an ODS guard column maintained at 25 °C. The mobile phase was methanol : 0.5 % formic acid aqueous solution (8/92, v/v) system in the gradient elution profile at a flow-rate of 0.8 mL/min, methyldopa was used as the internal standard (IS). The retention times of levodopa, benserazide and methyldopa were 3.9, 2.2 and 7.1 min respectively. The tandem MS optimal conditions were analyzed, and hypothetical electrospray ionization ESI(+)-MS/MS fragmentation pattern for levodopa, benserazide, dopamine and methyldopa was illustrated. Quantification was achieved using a positive electrospray ionization (ESI+) interface under multiple reaction monitoring (MRM) condition. No endogenous interferences were found for levodopa, benserazide and methyldopa, the matrix effects were negligible. The limit of detection (LOD) for levodopa and benserazide were 0.32 and 0.20 ng/mL in plasma, respectively. The calibration curve for levodopa showed linearity in the range 1.28-1280 ng/mL with a regression coefficient of 0.9997 and the calibration curve for benserazide showed linearity in the range 0.80-800 ng/mL with a regression coefficient of 0.9999. The pharmacokinetics of levodopa and benserazide was studied in Beagle dogs following intragastric administration. The $t_{1/2(\beta)}$ were 1.78 ± 0.24 and 2.28 ± 0.57 h, the T_{max} were 0.67 ± 0.14 and 0.75 ± 0.00 h, the C_{max} were 55.21 ± 13.92 and 0.02 ± 0.01 $\mu\text{g/mL}$, the $AUC_{0-\infty}$ were 74.60 ± 12.16 and 0.04 ± 0.01 h $\mu\text{g/mL}$, the MRT were 1.78 ± 0.06 and 2.61 ± 0.39 h for levodopa and benserazide, respectively. The novel method was validated and suitably applied to measuring of levodopa and benserazide in plasma samples and its oral single dose pharmacokinetics to Beagle dog. Compared with the methods reported in literatures before, the result showed excellent accuracy, specificity and sensitivity, suggesting its applicability to pre-clinical and clinical pharmacological research.

KEY WORDS: Benserazide, ESI-MS/MS, HPLC, Levodopa, Methyldopa, Pharmacokinetic.

* Author to whom correspondence should be addressed. *E-mail:* yangjingliu@yahoo.com.cn, glduan@shmu.edu.cn.

These authors contributed equally to this work.